

Dinesh Kumar Srivastava
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Pankaj Kumar *Editors*

Agricultural Biotechnology: Latest Research and Trends



Springer

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Ajay Kumar Thakur • Pankaj Kumar
Editors

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Dr. Prashant Bhatt

The book is dedicated to Dr. Prashant Bhatt, a renowned plant biotechnologist and mentor to many students, including one of the authors (DKS) and several leading contributors in plant biotechnology. Prashant's humility, kindness, empathy, and optimism were exemplary; his innovation and vision were inspirational; and his laughter and love of life were infectious and hard to ignore.



Born into a family rich in moral values and Ayurvedic knowledge, Prashant had a lifelong thirst for exploration and overcame all financial limitations. During his Ph. D. (1976) in Plant Developmental Biology from Gujarat University, he assembled a cytophotometer along with his junior colleagues, which won him the Hari Om Ashram Award for Young Scientists. During this time, he forged a lifelong partnership in science and at home with his classmate Daksha Mehta, who with her quiet, reasoning, calming presence countered Prashant's excitable personality.

Prashant was awarded the National Merit Scholarship from the Government of India to pursue studies abroad (1977–1981). He worked with an eminent developmental biologist Ian Sussex at Yale University, who planted the seeds of biotechnology in him. He later served as a Visiting Scientist at the USDA's Vegetable Breeding Laboratory in Charleston, South Carolina, for developing in vitro selection methods for herbicide resistance.

During his tenure as an Associate Professor at the MS University of Baroda, he teamed with Professor Atul Mehta making it a centre of excellence for plant tissue culture. Funding from CSIR and UGC, Government of India, attracted workshops at an international level and inspired young Indian scientists to pursue careers in plant biotechnology. He also conducted multidisciplinary research in collaboration with experts in Biochemistry and Horticulture, and taught courses at the Department of Microbiology and Genetics, introducing new curricula of somatic cell genetics.

With award of a Senior Fulbright Fellowship by USEFI, in 1986, he joined the laboratories of Professors David Hildebrand and Joe Chappell at the University of

Kentucky, with significant work on the regulation of HMG CoA Reductase, the key enzyme in the sterol biosynthetic pathway.

In the late 1980s, several tissue culture laboratories began opening up all over India, encouraged by the emerging need for quality products and new developments in horticulture. The Bhatt's joined one such company. Dr Bhatt, as a Vice President of Unicorn Biotek in Andhra Pradesh, set up the first commercial tissue culture company built with indigenous equipment and know-how in India. Under Prashant's expertise, tissue culture crops other than banana were introduced. Tissue culture Strawberry—limited to the cool and hilly areas could now grow anywhere: in non-hilly, non-temperate parts of India such as the arid regions of Gujarat.

Dr Bhatt's set up Sun Agrigenetics in 1999 in Vadodara, with the vision and mission to bring biotechnology to farmers' benefit in Gujarat. Sun Agrigenetics introduced tissue culture plants of a variety of crops beyond banana. Some of the 'firsts' tissue culture plants developed and commercialised were *Coccinea*, *Trichosanthes*, watermelon, lemon, Giant Reed. With his vision, Sun Agrigenetics became the sole company with dual recognition: NCS-TCP recognition as a commercial unit, as well as a DSIR recognised research centre from the Government of India. Development commercial protocols for elite date palm and red sandalwood were Dr Bhatt's dream projects, research grants from the Department of Biotechnology bringing them to reality. While Sun Agrigenetics was being set up, farmers in Gujarat were hardly aware of tissue culture and its benefits to the agriculture sector. With Prashant's efforts in directly interacting with farmers' groups on the field, 'tissue plants' soon became a colloquial term in the farmer community. Even while working in a commercial environment, Prashant emphasised education and continued to guide students through lectures, field visits, and advising research dissertations through Universities. Several research projects, a number of research articles of national and international repute, and elected life membership to the Plant Tissue Culture Association of India speak of his achievements. In 2015, he was awarded the Professor A.R. Mehta Memorial Lecture by the Indian Botanical Society, a fitting acknowledgement to his and Professor Mehta's long-term collaboration and friendship.

Prashant's legacy consists of the legion of students he trained, who now have their own trainees, as well as his two daughters and a grandson.

Preface

Biotechnology has emerged as an interdisciplinary science amalgamating molecular biology, genetics, biochemistry, microbiology, and chemical and process engineering. Advances in biotechnology in the last decade have resulted in the development of a number of powerful techniques, which have enhanced the well-being of human, environment, and society by targeting the use of various biological entities to result in useful products. The motivation to edit a comprehensive volume on current trends in biotechnology arose from the growing awareness of the recent advances in plant tissue culture, cell and molecular biology, genetics and applied breeding, biochemistry, food processing, and various “omics” technologies that all have boosted the pace of growth of this vibrant science. This book covers a wide range of applications of biotechnology. These include discussions on in vitro propagation technology, encapsulation technology, in vitro production of secondary metabolites, applications of somaclonal variations, and doubled haploids in agriculture, molecular breeding, and application of nanobiotechnology in agriculture. Separate chapters are dedicated to discussing the impact of various “omics” technologies including phenomics, genomics, proteomics, metabolomics, and bioinformatics in biotechnology research and their role in crop improvement. Some chapters describe the development perspective and acceptance level of transgenic crops worldwide. Separate chapters on RNA interference technology and miRNA-mediated regulation of biotic and abiotic stress responses in plants have also been discussed. Genome editing technology along with its probable applications has been discussed in detail.

This book is intended as a reference for plant/agricultural biotechnologists and plant molecular biologists. Also, it will serve as a comprehensive text for graduate and postgraduate level students in the department of plant biotechnology and plant molecular biology.

This book is the culmination of the efforts of several dignified researchers, scientists, and postdoctoral fellows who are very well-known and reputed figures in different frontiers of biotechnology research sector. We sincerely believe that this book will prove to be a useful contribution not only to science but also to the general public interest. We express our gratitude and appreciation to all the contributing authors who helped us tremendously with their time, critical thoughts, and suggestions to put together this peer-reviewed edited volume. While every effort was made to reach uniformity in style, the presented results and ideas and

organizational details of the chapters reflect the preferences of the respective authors. The editors are also thankful to the Springer Publication group and their team members for giving the opportunity to publish this book. Lastly, we thank our family members for their consistent support, understanding, encouragement, and patience during the entire period of this work.

Solan, India
Bharatpur, India
Solan, India

Dinesh Kumar Srivastava
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About the Editors



D. K. Srivastava has retired as Director Extension Education, prior to this he worked as Professor and Head in the Department of Biotechnology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India. He did his post-doctoral studies in the field of Plant Molecular Biology at the Institute of Molecular Genetics, USSR Academy of Sciences, Moscow, USSR and at Washington University, St. Louis, USA. He has 32 years of research experience and 29 years of teaching experience in the field of Plant Biotechnology and Molecular Biology. He had guided 32 students of M. Sc. and 9 students of Ph.D. for their research work. He has published 110 research papers in the journals of national and international repute, 38 research articles and contributed 12 chapters in the edited books, and also authored a book on biotechnology. He has delivered 45 invited lectures and attended 47 national and international conferences. His main area of research includes Plant tissue culture, Genetic transformation and Molecular characterization of plants. He is life member of various National and International Academic bodies/societies. He has received many awards for his scientific contributions including 'MS Swaminathan Award' by the Society of Plant Research, Meerut, India.



Ajay Kumar Thakur is presently working as Senior Scientist (Biotechnology) at ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan. He has obtained his graduation, PG and Doctoral degrees from Dr. Y.S. Parmar University of Horticulture & Forestry, Solan, H.P. He got induced into Agricultural Research Services (ARS) in 2008. He has published 40 research/review papers in various journals of International and National repute, authored one book 'Agricultural Biotechnology at a Glance', contributed 8 book chapters and 22 popular articles. He has developed high efficiency plant regeneration and genetic transformation protocols in a number of crops including *Populus ciliata*, *P. deltoides*, *Punica granatum*, *Capsicum annum* and *Cucumis sativus*. Dr. Thakur is associated with *Brassica juncea* improvement programme using biotechnological interventions from last 11 years. He has developed a core set of SSR markers for *B. juncea* genomics and is presently working on germplasm characterization and association mapping of various agronomically important traits in this oilseed crop. He has been granted with one Indian patent and associated in the development of a high yielding Indian mustard variety Giriraj, a white rust resistant Indian mustard genetic stock DRMR MJA 35, which is a *Moricandia* system- based cytoplasmic male sterile line of *B. juncea*, and a multiple disease (Alternaria blight, white rust and powdery mildew) resistant Indian mustard genetic stock, DRMRIJ 12-48. Dr. Thakur has received many awards from various societies and scientific organizations for his scientific contribution. He is also an elected Member of Plant Tissue Culture Association of India.



Pankaj Kumar is presently working as Assistant Professor (Biotechnology), Department of Biotechnology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India. Formerly, he has worked as SERB- National Post Doctoral Fellow (DST Young Scientist Scheme) at Council of Scientific & Industrial Research - Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India. Dr. Kumar is working on plant secondary metabolite enhancement through cell and tissue engineering

approaches; Identification of molecular cues linked with enhanced metabolites using comparative transcriptomics approach and also have experience on hydro-aero cultivation of medicinal plants for industrial importance. During Doctoral Studies; he (research team) has generated technology at Dr. Yashwant Singh Parmar University of Horticulture & Forestry Nauni, Solan for developing insect pest-resistant transgenic plants in economically important vegetable crops of cauliflower cv. Pusa Snowball, cabbage cv. Pride of India and broccoli cv. Solan Green Head (with *cryIAa* gene) for insect pest resistance. He has been awarded DST INSPIRE JRF/SRF Fellowship, Department of Science and Technology, Ministry of Science and Technology, Government of India for Ph.D. full doctoral program. He has qualified ICAR AICE-SRF (PGS), ICAR ASRB National Eligibility Test (NET). He has published 35 research/review papers in various journals of International and National repute, contributed 13 book chapters and 5 popular articles. Dr. Kumar has received many awards from various societies and scientific organizations for his scientific contribution i.e. Young Scientist Award Biotechnology-2018 by Society for Plant Research, Young Scientist Award -2019 by the Society of Tropical Agriculture, New Delhi, India, Excellence in Research Award -2019 by Agro Environmental Development Society, India. etc.



Commercial Micropropagation of Some Economically Important Crops

1

Daksha Bhatt

Abstract

Commercial tissue culture commenced in India in 1984 subsequent to the laudable classic work at University of Delhi using basic tissue culture for studies in developmental biology commenced way back in the 1950s, and its importance gradually recognized for application in crop improvement. Since then it has been a trend of 'rise and fall, and rise again' till date. The total tissue culture plant production in India is currently estimated to be 300 million plants per annum, of which banana shares approximately 50%. Scores of species in fruit, vegetable, floriculture, forest, plantation, medicinal, and aromatic plant categories are now produced through micropropagation. Next to banana, sugarcane and ornamentals, other crops such as pineapple, pomegranate, lemon, ivy gourd (*Coccinea indica*), pointed gourd (*Trichosanthes dioica*), and teasle or spine gourd (*Momordica dioica*), turmeric, and ginger are now commercially produced through micropropagation in India. There are challenges for the micropropagation industry producing banana and other plants in general, with many tissue culture units facing severe economic loss ending up in non-performance or closure. Some aspects of the problems are discussed in detail.

Keywords

Commercial micropropagation · Banana · Cucurbits TC · Micropropagation challenges

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1.1 Introduction

The laudable efforts by Late Professor PM Maheshwari in year 1950 for using tissue culture techniques for basic studies are the keystone for modern plant biotechnology in India. The discovery of haploids in tissue culture by Guha and Maheshwari (1964) became a global landmark in the field of developmental biology. It was later in the 1960s when another tissue culture laboratory was set up at the MS University of Baroda, where apart from developmental studies, practical aspects of tissue culture began to be considered under the able guidance of Late Professor Mehta. Plant tissue culture remained limited to an academic interest until its commercialization was incepted in early 1984, with *A.V. Thomas Co.* beginning cardamom tissue culture (TC) for domestic use. The stage was set by them for ornamental crops, produced for export purpose. Very soon other companies like *Indo American Hybrid Seeds* joined the scene to produce tissue culture plants of ornamentals and also initiated work on banana tissue culture. Ornamental plants remained at the fore-front then with an idea to reach the European export markets. No one would have perhaps had the hint at that time that horticulture crops were beckoning, calling for a very different picture decades later for commercial micropropagation in India.

1.2 The Beginning

Several commercial plant tissue culture (PTC) units were soon set up in the later part of the 80s, with imported equipment and technology collaboration. The banana was the first candidate among the many horticultural crops that were identified. The first indigenous commercial laboratory was set up in Hyderabad with in-house technology development at *Unicorn Biotech Ltd* in 1987 under the guidance of Late Dr. P. Bhatt. During this period, more than 50 commercial laboratories were set up, with banana and sugarcane as the main crops and a high total installed capacity of around 210 million plants per annum. Department of Biotechnology (DBT), Government of India (GOI), set up two Tissue Culture Pilot Plants in 1989, one of them being at TERI's in Haryana. Within the next 2 years, the number of commercial units went up to 74. In Maharashtra, 25 units were set up. There were nine PTC units each in Karnataka and West Bengal, 6 units in Andhra Pradesh, 4 each in Tamil Nadu and Kerala, 3 each in Gujarat, Haryana and UP (Govil and Gupta 1997). At that time around 75 species in ornamental (foliage plants, flowering plants), fruit, vegetable, plantation crops, and forest tree species were produced by these laboratories.

Resistance to accepting tissue culture plants by small growers, inconsistencies of production know-how resulting in poor performance, narrow product range, lack of trained manpower and infrastructure were the chief constraints that these leaders initially faced resulting in fluctuated outputs and capacity utilization gradually dropping to around 50%. For them, there were no guidelines to follow. The scenario gradually changed in later years after 1991 when GOI identified micropropagation of plants as an industrial activity under the Industries (Development and Regulation) Act of 1951, which gave a boost to the commercialization of plant tissue culture

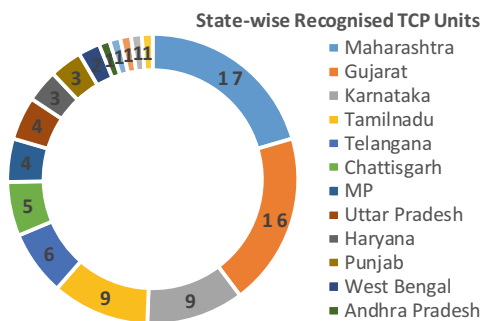
(PTC). In 2005, only 45 PTC units were functioning with production volumes of 30 million plants per annum (Shukla et al. 2012). GOI, through DBT, formed the National Certification System (NCS-TCP) in 2006 to establish a structured system for production and distribution of quality planting material, and to regulate its commercial production. Since its inception, there is a significant impact on the quality of plants produced, as evident from the fact that there has been no major virus outbreak (DBT 2016). With these guidelines and intervention by the GOI, again there was a rise in the production of tissue culture plants (TCP) mounting to a capacity to 300 million plants per annum in the year 2012. In 2016, there were 100 NCS-TCP recognized units; with units not yet recognized, the aggregate score was 200. The actual production of TC plants was 350 million in total installed capacity of 500 million (Shukla 2017). Thus, the history of commercial tissue culture in India is a story of ‘rise and fall, and rise again’ as quoted by Prakash (2006).

1.3 Present Scenario

In the past two decades, the plant micropropagation industry has made a very strong presence in line with other biotech industries. As of date, there are 83 NCS-TCP recognized production units spread across the country. Out of these, the first four states: Gujarat, Maharashtra, Tamil Nadu, and Karnataka house 51 units, that is, 61% of total PTC units (Fig. 1.1). Capacity wise, some PTC units are large with installed capacities of around 1–3 million plants/annum. Jain Irrigation Ltd. set up their PTC unit in 1995—today it is the largest PTC unit in the world with a production capacity of 70 million plants per annum producing banana, pomegranate, and strawberry. Others have smaller capacities between 0.5 and 1 million. In 2016, the gross installed production capacity was about 500 million plantlets per annum, as of the date this number would have increased by 25%. The industry witnesses an actual production of approximately 350 million plants (DBT stakeholders meet 2016). Banana, potato, sugarcane, apple, pineapple, strawberry, gerbera, anthurium, liliun, orchids, bamboo, date palm, teak, and pomegranate are some of the major plants tissue cultured in India. Cardamom TCP covers 500 ha and vanilla covers 50 ha among the spice group plants (Anis and Ahmad 2016). Apart from this, turmeric, ginger, lemon, pointed gourd, ivy gourd, spine gourd, and papaya are new additions to the product list of TCPs.

The role of various plant tissue culture techniques in new opportunities for its contribution towards crop improvement, productivity, and food security has been reviewed by several workers. In the earlier years, ornamental plants were the main TC product of some of the commercial companies in trend with the global market, and this was reviewed in detail by Govil and Gupta (1997). Prakash (2000) enumerated the factors influencing the development of the micropropagation industry in India and explained some management and planning issues that hampered further growth of the industry. Later on, they analysed the opportunities and

Fig. 1.1 Present state-wise distribution of NCS-TCP recognized PTC units (DBT 2020)



problems faced by the industry (Prakash 2012). Recently, Ragavendran and Natarajan (2017) presented an extensive review of TC in all crop species for fast multiplication, the introduction of new traits in crop plants and the development of crops resistant to biotic and abiotic stress, signifying the value of this technology in food security at a global level. Anis and Ahmad (2016) highlighted that in order to make commercialization successful for any crop, the technology should be reproducible and the system must have the least chances of genetic instability. Kumar (2017) in his review has focused on banana PTC industry in particular, mentioning the need to reduce the demand–supply gap with better marketing policies and better distribution of certified quality plantlets to farmers. Gulzar et al. (2020) described in detail various applications of plant biotechnology for genetic improvement and illustrated the viability of transgenic methods for clonal production of valuable germplasm. Whereas many of the reviews and reports signify opportunities and applications of PTC and results are mainly limited to laboratory, pilot-scale or field trials, a clear picture does not emerge on its further application by the PTC industry in terms of absolute volumes produced for each crop and what is the current trend in commercialization, barring the TC banana. In the absence of authentic information, it is only an estimate that can be made on total production for each type of product based on market information and personal communication with concerned people. Based on this, the major producers (albeit not limited) of PTC in the country are shown in Table 1.1.

Among banana, Grand Naine, a popular variety grown mostly in all export-oriented countries of Asia, Africa, South America, is a superior selection of Giant Cavendish which is introduced in India since 1990. Due to many desirable traits like excellent fruit quality, immunity to fusarium wilt, it has proved a better variety (Singh & Chundawat 2002). India being the largest producer of banana, and the need for large volumes of planting material, it is not surprising that it was the first horticultural crop to be commercially produced through TC. The first PTC banana was incidentally sold at a price twice the current rate in the 1980s by Indo American Hybrid Seeds when the very first TC banana was produced. As evident from

Table 1.1 List of NCS-TCP recognized units (DBT 2020), with total production volumes (wherever available) in the past year, and some non-recognized units (personal communications)

State	Total recognized units in state	S. no.	Name of unit	Products	PTC units not recognized	Products
Andhra Pradesh	1	1	Micco Laboratories Pvt. Ltd.			
Bihar	1	2	Hecure Agro Plants Pvt. Ltd.			
Chhattisgarh	5	3	Aditya Biotech Lab & Research Pvt Ltd.	Banana, Jatropha, Acacia, Eucalyptus, gerbera, sugarcane, bamboo		
		4	AKF Plant Sciences Pvt Ltd.	Banana, ginger, pomegranate	Mahaveer Iron and Steel Ltd. (plant tissue culture division)	Banana, gerbera, ornamentals, strawberry
		5	COE AIB Tissue Culture Lab, IGKV			
		6	Devleela Biotech	Banana, bamboo		
		7	Yash Biotech, Raipur	Banana, bamboo, sugarcane		
Gujarat	16	8	ABC Agrobiotechnology Pvt. Ltd.	Banana, lemon, teak, guava, pomegranate, potato, bamboo	Vasundhara biotech	Banana
		9	Aranya Agri Biotech LLP	Banana, pointed gourd, ivy gourd, teale gourd, lemon, pineapple, fig, pomegranate	Kalpataru Agro Biotech	Banana
		10	Greenfield Biotech	Pointed gourd, banana, <i>Coccinia</i>	Sri Ratnam Biotech,	Banana
		11	IRM Enterprises Pvt. Ltd. (formerly Cadila)		Shri Abhimanyu Biotech	Banana

(continued)

Table 1.1 (continued)

State	Total recognized units in state	S. no.	Name of unit	Products	PTC units not recognized	Products
			Pharmaceuticals Ltd.- Agro Division)	Banana, potato, lily bulb, foliage, pointed gourd, teak, sugarcane, pomegranate		
		12	Kutch Crop Services Ltd.	Banana	Ambika Agro	Banana, pointed gourd
		13	Metrogen Biotech		Gangamani Agri Biotech	Banana, pomegranate
		14	Natural Life Sciences	Banana, potato, sweet potato, fig, lemon, pineapple, pointed gourd, ivy gourd		
		15	PAC Bio Fungbact Pvt. Ltd.	Banana		
		16	Palaj Agrotech	Banana		
		17	Sarjan Biotech Pvt. Ltd.	Banana, pomegranate		
		18	Shaili Biotech (P) Ltd.	Phormium, dracaena, banana, sarracenia, alpinia dionaea, lily nepenthes		
		19	Siddhi Plantek	Banana, bamboo pomegranate, potato, pointed gourd, lemon		
		20	Shree Ganesh Khand Udyog Sahakari Mandali Limited	Sugarcane, Banana		
		21	UMA Biotech Industries	Banana		
		22	Vasundhara Agribiotech	Banana		
		23	Virigold Biotech Pvt. Ltd.	Banana		

Haryana	3	24	Sheel Biotech Ltd.	Banana, citrus, apple, gerbera,, lilium, anthurium, gladiolus, bamboo, eucalyptus, poplar, Yucca		
		25	Technico Agri Sciences Ltd.	Potato		
		26	Tata Energy Research Institute (TERI)	Sandalwood plants, teak (Sagwan) plants, red sandalwood, bamboo, banana, sugarcane, potato, papaya, pineapple, strawberry, turmeric, ginger, syngonium, ficus, spathiphyllum, anthurium, cordyline		
Himachal Pradesh	1	27	Nishant Biotech	Apple, cherry and pear rootstocks, kiwi		
Karnataka	9	28	Biotechnology Centre, Dept. of Horticulture		EK Plant technologies	Ornamentals
		29	H.U. Gule Biotech Pvt. Ltd.	Banana	Excel Plant Link Pvt. Ltd.	Banana, pineapple, ornamentals, carnation, gerbera, gypsophila, strawberry, phalaenopsis
		30	Hybrid Agri Biotech Pvt. Ltd.	Banana	Meghana Tissue Culture Nursery	Banana
		31	Jagadamba Bio Plants	Banana, teak, pomegranate, date palm, potato, ornamentals, medicinal, bamboo, cardamom, vanilla	Unique Plant Tech and Nursery, Karnataka	Banana, ornamentals, medicinal plants

(continued)

Table 1.1 (continued)

State	Total recognized units in state	S. no.	Name of unit	Products	PTC units not recognized	Products
		32	K F Biotech Pvt Ltd.		Aditya biotech	
		33	Mysore Organic Farms Pvt. Ltd.	Banana		
		34	Novel Biotech			
		35	Shanthi Agrotech	Banana		
		36	Sree Adithya Biotech			
Maharashtra	17	37	Ajeet Seeds Ltd.	Banana	A1 Biotech	Banana and house plants
		38	Beejsheetal Research Pvt Ltd.	Banana		
		39	Biosis Plants Pvt. Ltd.	Banana, strawberry	Bhoomiputra Biotech	Banana
		40	Geeta Agro Biotech	Banana	Jain Irrigation Systems Ltd.	Banana, pomegranate, strawberry
		41	H.U Gugle Agro Biotech Co.	Banana		
		42	Ishved Biotech Pvt. Ltd.	Horticultural, ornamental and floriculture plants	Ecofriendly Biotech and Bioplants Pvt. Ltd.	Banana, gerbera, carnation
		43	Janani Biotech and Tissue Culture Lab		Shri Ganesh Biotech	Banana
		44	K.F. Bioplants Pvt. Ltd.	Gerbera, rose, carnation, lily strawberry, limonium, alstroemeria, gypsophila, ranunculus, phalaenopsis	Kisan Agri Biotech	Banana
		45	Kimya Biotech Pvt. Ltd.	Gerbera, carnation	Nirmal Seeds P.Ltd.	Banana, pomegranate

		46	Kshitij Biotech Corporation	Banana, ginger, date palms, turmeric	Patil Biotech	Banana
		47	Mahabeej Biotechnology Centre			
		48	Namo Bioplants			
		49	Ram Biotech			
		50	Rise N Shine Biotech Pvt. Ltd.	Banana, gerbera, carnation, orchid, chrysanthemum	Callus Biotech	Banana, Santalum
		51	Seven Star Fruits Pvt. Ltd.			
		52	Thopte Biotech Pvt. Ltd.			
		53	Vasant Tissue Culture Laboratory	Banana		
MP	4	54	Reva Flora Culture	Bamboo, Banana, pomegranate, teak, lemon, etc.	ITI Biotech Tissue Culture Lab	<i>Banana, Roses, Stevia, Gerbera, Anthurium, Parwal, guava, Citrus, strawberry</i>
		55	Sachdev Nursery	Pomegranate, lemon, guava, orchid, banana, sugarcane	Bhoomi Biotechnology Venture for Research and Development	Pomegranate, Banana, bamboo
		56	Shri Mukund Biotech			
		57	Tirupati Fresh Agro Crop Science Pvt. Ltd.	Banana, pomegranate, sugarcane		
Odisha	1	58	Excel Plant Link Pvt. Ltd. (UNIT-II)			
Punjab	3	59	Bhatti Tissue Tech.	Potato	Bharat Agritech	Potato
		60	Mahindra H2pc Pvt. Ltd.	Potato, banana		

(continued)

Table 1.1 (continued)

State	Total recognized units in state	S. no.	Name of unit	Products	PTC units not recognized	Products
Rajasthan	1	61	PepsiCo India Holding Pvt. Ltd.			
		62	Atul Rajasthan Date Palms Ltd.	Date palm		
Tamil Nadu	9	63	Annai Meenashi Biotech			
		64	Genewin Biotech	Syngonium, banana, philodendron xanadu, orchids, vanilla, gerbera, bamboo, aloe, pomegranate		
		65	Growmore Biotech Ltd.	Over 50 crops: fruit crops, ornamentals, commercial, trees and medicinals		
		66	Hi-Fi Biotech India Private Limited	Banana		
		67	Hosur Hortitech	Banana		
		68	Jay Blossoms Bio Tech	Banana		
		69	Jayasree Biotech, Hosur	Banana		
		70	SPIC Agro Biotech Centre	Banana, gerbera		
		71	Sree Visal Biotech			
Telangana	6	72	ACE Agro Technologies	Banana, date palm	Greeno Agrotech India	Banana, pomegranate, fig philodendron, xanadu, cordyline, teak, bamboo
		73	Agri Vitro Tech Laboratories	Banana, Gerbera		Teak and others

							Shivshakti Biotechnologies Ltd.		
		74	Atlantis Phyto Tech	Banana			Sri Soam Biotech	Banana	
		75	Kisan Agri Biotech, Hyderabad				Sri Venkateshwara Agro Technologies	Banana	
		76	Microsun Bioplants (India) Pvt. Ltd.	Banana			Tulasi Biotech		
		77	Rodasy Biotechnologies	Banana			Visal Agri Biotech		
Uttar Pradesh	4	78	Dr. MC Saxena Group of Colleges	Banana					
		79	Hindustan Bioenergy Ltd.						
		80	Sagar Agrisciences Pvt. Ltd.	Banana					
		81	Sagar Agrisciences Pvt. Ltd. (UNIT-II)	Banana					
West Bengal	2	82	Elegant Flower Company Pvt. Ltd.	Potato, banana			Sristi Agro Biotech Pvt. L. (W Bengal)	Banana, house plants, flowers, strawberry, bamboo, vanilla, cardamom, rhododendron	
		83	Pallishree Ltd.	Banana, pineapple, gerbera, orchid, syngonium, philodendron, Xanadu			Synergy Agri Products P. Ltd. (W Bengal)	Banana, alocasia, calathea, yucca Cordyline australis atro, elephanitipes	
Kerala							AVT Biotech	Banana, leek, wasabia, ornamentals	
							Greenvalley Biotech	Banana, sugarcane, ornamentals	

statistical data of the past 5 years, the area under production of banana is on a continuous rise (Tables 1.2 and 1.3).

The estimated production of banana in year 2016–2017 was 31.08 million tons (NHB 2017), which rose to 3.7% higher to 31.17 million tons in the subsequent year, and 10% higher than the previous 5 years' average (Press Information Bureau 2019). Thus, there is a steady increase in production as well as productivity within the country. India contributes up to 26% of total world production of banana having higher productivity than the world average, but stands lower compared to the productivity of Indonesia (Fig. 1.2). Within the country, six states (Gujarat, Tamil Nadu, Andhra Pradesh, Gujarat, Maharashtra, and UP) contribute 2/3 of the production from 1/2 of total area (Fig. 1.3). Commercial cultivation of banana is not only limited to the plains in the major banana growing states. TC banana has also reached the States of Jammu (Jeet et al. 2019), as well as in Meghalaya (Fig. 1.4a), from where the local clones of Hill banana were procured, multiplied through micropropagation and cultivated (Meghalaya SFAC 2014); Dir of Horticulture Meghalaya, personal communication).

With total area under banana cultivation of 859,000 ha, 2.546 billion plants are required at the rate of 2964 plants/ha. The current total annual TC banana plant production is estimated (Table 1.1) to be around 150 million. Out of this, the four states in South India- Telangana, AP, Karnataka, and Tamil Nadu produce 11–14 million banana each year (Rao TVLN, Mane R, personal communication). Report from Rabobank (2019) gives an official estimate of TC banana production in India up to 80 million plants which would be about 3% of total requirement of planting material. In another publication (APAARI 2019), it is stated that 17% of banana plantations in India are TC derived. In this case, TC banana produced would be 432 million. If this estimate is correct, the un-recognized labs are producing a much larger volume of TC banana than those that are DBT recognized. All these are calculated estimates, but it is clear that the market share of TC banana plants stands in-between 3 and 17%. The Department of Biotechnology in India expects the annual growth in demand for tissue-cultured banana plants to increase at a rate of 25%.

Whatever are the statistics, being no more than estimates or assumptions, it is very clear that we are still lagging in productivity and the need for quality planting material *inter alia* is still looming over on modern horticulture, as brought to our attention from frequent reports from National Research Centre for Banana and other publications. The productivity of banana in India is higher than world average, like in few other crops (Fig. 1.2), but still lower than world's best productivity. A significant control has been brought over due to disease free TC banana and the dominance of Cavendish banana which are resistant to *Fusarium oxysporum* f. sp. *cubense*. A new threat has arisen since the past couple of decades from a new race of *F. oxysporum*, called TR4. Its presence is confirmed in Asia, South Africa, and Australia. FAO (2020) goes to state that further spread of TR4 would have a significant impact on global banana production and business. It can infect and destroy plants that were resistant to the older races of *Fusarium*. This includes Cavendish and other varieties as well as plantains. So far, no methods of eradication

Table 1.2 The area under total cultivation of some crops (only those crops where commercial TC production is done are included) in India (NHB 2018)

Area of cultivation (million ha)	Year		Production ('000 MT)							
	2014–2015	2015–2016	2016–2017	2017–2018	2014–2015	2015–2016	2016–2017	2017–2018	2016–2017	2017–2018
crop/year										
Banana	0.822	0.841	0.860	0.884	28.1	29.1	30.4	30.8	30.4	30.8
Potato	2.14	2.12	2.18	2.14	51.3	43.4	48.6	51.3	48.6	51.3
Pineapple	0.10	0.11	0.11	0.103		1924	1861	1706		
Lemon	0.268	245	248	286	2950	2438	2364	3148		
Papaya	0.115	0.132	0.134	0.138	4913	5667	5940	5989		
Pomegranate	0.181	0.197	0.209		1789	2306	2613	2845		
Turneric	0.184	0.197	0.216	0.234	830	943	1052			
Parwal	NA	0.018	0.018	0.020	NA	18	18	20		
Other gourds				0.110				660		

Table 1.3 Estimates of production and productivity in various crop categories for 2018–2019 and advance estimates for 2019–2020 (GOI Press Release 2020)

Crops	2018–2019		2019–2020 (first advance estimate)		2019–2020 (second advance estimate)	
	Area (in '000 ha)	Production '000 MT	Area (in '000 ha)	Production '000 MT	Area (in '000 ha)	Production '000 MT
<i>Fruits</i>						
Apple	308	2316	308	2734	308	2734
Banana	866	30,460	875	29,649	878	31,504
<i>Citrus</i>						
Lime/lemon	305	3482	314	3547	317	3717
Guava	276	4253	286	4345	287	4304
Pineapple	104	1711	108	1781	107	1799
Pomegranate	253	2915	264	2329	261	2315
Strawberry	1	5	1	8	1	8
Others	248	2298	255	2281	255	2268
Total fruits	6597	97,967	6660	95,743	6664	99,069
<i>Vegetables</i>						
Parwal/pointed gourd	55	757	55	741	56	760
Potato	2173	50,190	2149	51,947	2158	51,300
Sweet potato	110	1156	116	1194	116	1186
Tapioca	163	4976	139	4046	164	5043
Others	1441	21,118	1519	21,156	1504	22,962
Total vegetables	10,073	183,170	10,292	188,009	10,353	191,769
Aromatics and medicinal	627	795	634	822	628	798
Flowers cut		647		688		762
Flowers loose	303	2263	294	2186	305	2301
Total flowers	303	2910	294	2873	305	3063
Total plantation	3872	16,350	3866	16,412	3887	16,240

<i>Spices</i>										
Cardamom	81	23	79	26	79	26	79	26	79	26
Garlic	358	2910	356	2862	363	2862	363	2917	363	2917
Ginger	164	1788	168	1805	172	1805	172	1844	172	1844
Vanilla	0	0.1	0	0	0	0	0	0	0	0
Saffron	3	0	3	0	4	0	4	0	4	0
Turmeric	253	961	239	913	246	913	246	939	246	939
Total spices	3960	9428	3866	9372	3824	9372	3824	9420	3824	9420
Total	25,433	310,738	25,611	313,351	25,661	313,351	25,661	320,479	25,661	320,479

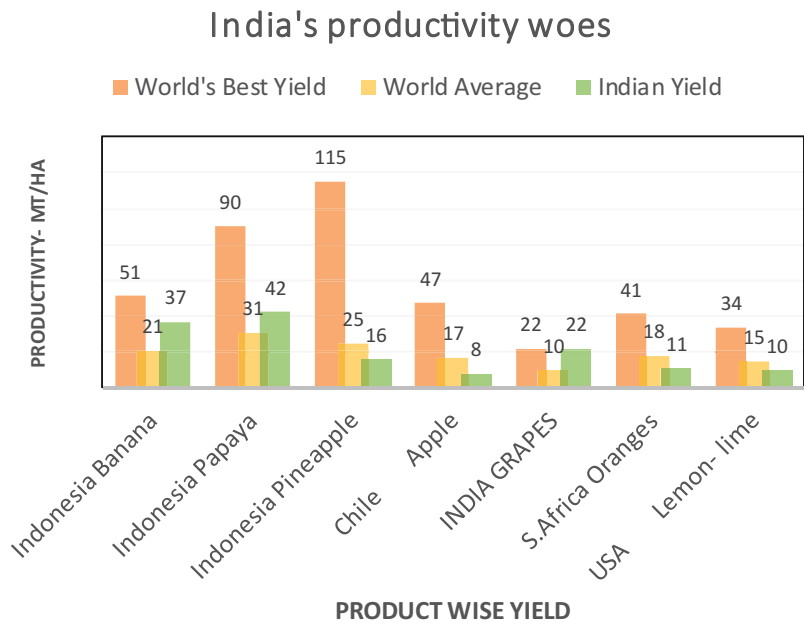


Fig. 1.2 Crop yields in major countries with maximum and average yields

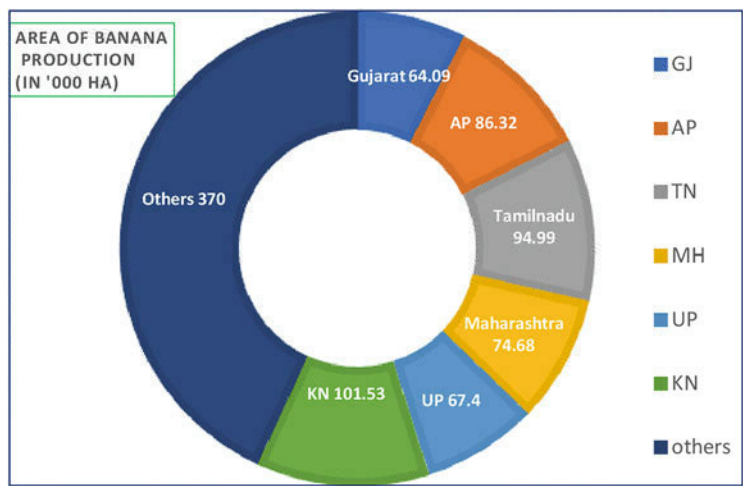


Fig. 1.3 State-wise area under cultivation of banana (in '000 ha) in different states of India (Data from NHB 2017)

are available (Hwang 2001). Approaches have been made to look for resistance in wild genotypes or by mutation breeding with some success (Smith et al. 2006). Somaclonal variation has been exploited in micropropagated plants for various